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Adenylate cyclase and cell cyclic AMP of rat cortical thick ascending limb of Henle

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Adenylate cyclase and cell cyclic AMP of rat cortical thick ascending limb of Henle. Effects of parathyroid hormone (PTH), calcitonin (CT), vasopressin (VP), and glucagon (GL) on adenylate cyclase activity and cyclic AMP (cAMP) levels in isolated cortical thick ascending limbs of Henle's loop (CTAL) of the rat kidney were examined. PTH, CT, and VP each stimulated adenylate cyclase of this nephron segment in a dose-dependent manner. Stimulation of the enzyme activity was greatest with a maximal dose of PTH and least with VP. With maximal doses, the effects of PTH and CT were not additive; whether or not the effects of maximal doses of VP and PTH or CT were additive was not clear. All three hormones increase cAMP in intact CTAL in a dose-dependent manner. Maximal doses of PTH, CT, VP, and GL resulted in comparable rises in cell cAMP, and there was no additive effect. These data suggest that PTH and CT may stimulate the same adenylate cyclase moieties, whereas VP may stimulate distinct enzyme moieties, and that these three peptide hormones as well as GL definitely act on the same cell group in rat CTAL. Thus, it is possible that these hormones may induce qualitatively similar effects on CTAL functions if such effects are mediated by cAMP.

Adénylate cyclase et AMP cyclique cellulaire de la branche ascendante large de l'anse de Henle chez le rat. Les effets de l'hormone parathyroïdienne (PTH), de la calcitonine (CT), de la vasopressine (VP) et du glucagon (GL) sur l'activité adénylate cyclase et la concentration de cAMP dans des anses ascendantes larges corticales de Henle (CTAL) du rein de rat ont été étudiés. La PTH, la CT, et la VP stimulent individuellement l'adénylate cyclase de ce segment d'une façon dépendante de la dose. La stimulation maximale de l'activité enzymatique est la plus grande avec la PTH et moindre avec VP. A concentrations maximales, les effets de la PTH et de la CT ne sont pas additifs; il n'est pas clairement établi que les effets de concentrations maximales de VP et de PTH ou de VP et de CT sont ou non additifs. Les trois hormones augmentent cAMP dans le CTAL intact d'une façon dépendante de la concentration. Des concentrations maximales de PTH, de CT, de VP, et de GL déterminent des augmentations de concentration comparable de cAMP cellulaire et il n'y a pas d'additivité. Ces résultats suggèrent que la PTH et la CT pourraient moduler les mêmes fractions d'adénylate cyclase alors que la VP stimulerait une fraction distincte, et que ces trois hormones peptidiques de même que GL agissent sur le même type cellulaire du CTAL du rat. Ainsi il est possible que ces hormones puissent induire des effets qualitativement similaires sur les fonctions du CTAL si ces effets ont le cAMP pour médiateur.

Each nephron segment has distinct transport characteristics and hormone sensitivity. The distribution of adenylate cyclase's sensitivity to parathyroid hormone (PTH), calcitonin (CT), vasopressin (VP), and beta adrenergic agonists has been described by the ultramicroassay of adenylate cyclase in isolated single nephron segments of rabbits, mice, rats, and of man [1–7]. In the thick ascending limb of Henle's loop (TAL), PTH has been shown to stimulate adenylate cyclase activity in the cortical portion (CTAL) of rabbit, mouse, rat, and human kidneys, whereas this hormone has no effect on the enzyme activity of the medullary portion (MTAL) of rabbit, mouse, and rat kidneys [6]. By contrast, CT stimulates the enzyme activity in both MTAL and CTAL of rabbit and human kidneys, but it has minimum effects on adenylate cyclase of mouse CTAL and MTAL [7]. VP also stimulates the enzyme activity of both MTAL and CTAL of mouse kidney and to a lesser extent that of both segments of rabbit kidney [7]. In addition, a recent study showed the presence of glucagon (GL) -sensitive adenylate cyclase in CTAL of rat kidney [8]. Although the physiologic significance of these hormones in TAL is not known, adenylate cyclase's sensitivity to more than one hormone in one nephron segment raises a possibility that these hormones may exert similar biological effects if such effects are mediated by cAMP.

To entertain such a possibility, one must know whether these hormones act on the same adenylate cyclase moiety and on the same tubular cells. Effects of multiple agonists on adenylate cyclase activities will provide information on whether or not they act on the same or different enzyme moieties: If effects of hormones at maximal concentrations are nonadditive, it follows that they stimulate the same enzyme moiety and thus must act on the same cells. If multiple agonists act on distinct cell groups, their effects on cell cAMP contents must be additive, whereas if they act on the same cell group, the effects are either additive or nonadditive. Thus, the lack of additive effects will provide useful information [7]. Accordingly, we examined the effects of PTH, CT, VP, and GL on adenylate cyclase activity and on the levels of cell cAMP in rat CTAL.

Methods

Wistar male rats weighing 100 to 300 g were used in the present study. Hormones used were parathyroid hormone (PTH; 1–34 amino acids bovine synthetic PTH, 6,000 U/mg, Beckman Co., Palo Alto, California), synthetic salmon calcitonin (CT; 4,000 U/mg, Armour Pharmaceutical Co., Scottsdale, Arizona), synthetic arginine vasopressin (VP; grade VI, Sigma

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Chemical Co., St. Louis, Missouri), and glucagon (GL; Eli Lilly & Co., Indianapolis, Indiana). In some experiments, parathyroid extract (PTE; Parathyroid Injection USP, Eli Lilly & Co., Indianapolis, Indiana) was used.

Preparation of isolated single nephron segments and assay of adenylate cyclase activity. Rats were anesthetized by i.p. injection of pentobarbital, 40 mg/kg body wt. The right renal and superior mesenteric arteries were ligated, and the left kidney was perfused, through a PE-100 catheter inserted in the abdominal aorta below the renal arteries, over a few seconds with 5 ml of chilled Krebs-Ringer-bicarbonate (KRB) buffer: 105 mM sodium chloride, 5 mM potassium chloride, 25 mM sodium bicarbonate, 10 mM sodium lactate; 1.0 mM monobasic and dibasic sodium phosphate, 1.0 mM magnesium chloride, and 1.0 mM calcium chloride (pH 7.4) with 8.3 mM glucose, equilibrated with 95% oxygen and 5% carbon dioxide (vol/vol) followed by a rapid perfusion over 5 to 10 sec with 10 ml of ice-cold KRB buffer containing 8.3 mM glucose, 10 mg of collagenase (type I, #69C-0354, Sigma Chemical Co., St. Louis, Missouri), and 10 mg of bovine serum albumin (BSA; KRB-BSA-collagenase). Immediately before the perfusion of the left kidney, the aorta was clamped above the left renal artery.

The left kidney was removed, and slices of kidney tissue (approximately 0.5 to 1.0 mm in thickness) cut in the corticomedullary plane were incubated at 30° C for 30 min in KRB-BSA collagenase with 95% oxygen and 5% carbon dioxide (vol/vol) as a gas phase. Kidney slices were rinsed with 50 ml of ice-cold, calcium-free Hanks' solution: 137 mM sodium chloride, 5.3 mM potassium chloride, 0.34 mM dibasic sodium phosphate, 0.44 mM monobasic potassium phosphate, 4.2 mM sodium bicarbonate, 0.42 mM magnesium sulfate, and 0.49 mM magnesium chloride (pH 7.4). Tubule segments were dissected from cortex (CTAL) and from outer medulla (MTAL) under the stereomicroscope in an ice-cold, calcium-free Hanks' solution. Each dissected tubule segment was transferred to a siliconized individual well of a serological slide, and the tubule length was measured with an aid of a drawing tube attached to the microscope (Wild M5 dissecting microscope). Adenylate cyclase activity in isolated nephron segments was assayed by the method described by Imbert et al [1] with modifications described earlier [9]. After careful aspiration of the dissecting medium bathing the tubules, 1 μl of hypotonic preincubation solution (8 mM Tris hydrochloride buffer (pH, 7.4), 1 mM magnesium chloride, 0.25 mM EDTA-sodium, and 0.1% BSA) was added. This preincubation solution contained appropriate test hormone(s) or vehicle at concentrations five times greater than those indicated in the results as final concentrations [1–3]. After 30 min of preincubation at 0° C, tubule segments were freeze-thawed twice. Then, 4 μl of incubation medium was added consisting of 100 mM Tris hydrochloride buffer (pH, 7.4), 3.8 mM magnesium chloride, 0.25 mM EDTA-sodium, 1.35 mM 1-methyl-3-isobutylxanthine (MIX), 0.6 mM ATP, 20 mM phosphocreatine, and 1 mg/ml creatine phosphokinase (140 U/mg, Sigma Chemical Co., St. Louis, Missouri), and the incubation was carried out at 30° C for 30 min.

The incubation was terminated by the addition of 10 μl of 0.2 N hydrochloric acid followed by the successive addition of 10 μl 0.2 N sodium hydroxide and 200 μl of 50 mM sodium acetate buffer (pH, 6.2). An aliquot of the resultant mixture was assayed for cAMP, after acetylation of cAMP, by the radioimmunoassay (RIA) using a highly specific antibody toward succinyl-cAMP as reported earlier [9]. In brief, RIA was performed in 200 μl of 50 mM sodium acetate buffer (pH, 6.2), containing approximately 10,000 cpm ^{125}I-cAMP (specific activity, about 250 Ci/mmole; New England Nuclear, Boston, Massachusetts), rabbit anti-succinyl cAMP antibody prepared in our laboratory diluted to bind 50 to 60% of ^{125}I-cAMP in the absence of added cold cAMP, and standard cAMP or samples both acetylated immediately prior to assay. After overnight incubation at 4° C, the cAMP-antibody complex was separated by adding 60% ammonium sulfate followed by a centrifugation at ×2,000*g* for 25 min at 4° C. Significant displacement of ^{125}I-cAMP was detectable with the addition of 10 fmoles of nonlabeled cAMP, and the standard curves were linear between 10 and 500 fmoles of nonlabeled cAMP added and plotted on the semi-log scale. Samples were accordingly prepared to contain 10 to 500 fmoles of cAMP per assay. The accuracy of RIA was checked by (*1*) the linearity of assays after serial dilutions of samples, (*2*) the complete disappearance of the immunoreactivity by phosphodiesterase treatment, (*3*) the lack of effects on RIA of any component of the incubation mixture at concentrations used, and (*4*) linear positive correlations ($P < 0.01$) between the tubular length and cAMP formed. Further, the purification of cAMP by column chromatography followed by the precipitation of zinc sulfate and barium hydroxide [10] gave comparable values of cAMP determined by the RIA.

Results were expressed as femtomoles of cAMP formed per millimeter of tubule length per 30 min.

Incubation and assay of cyclic AMP in intact tubule segments. Modified Hanks' solution containing 1.0 mM calcium chloride, 1.2 mM MIX, and 0.25% BSA was used for both dissection and incubation of isolated nephron segments. In some experiments, MIX was either omitted or reduced to 0.12 mM. Two to five single nephron segments (total length, 2 to 6 mm) were transferred in 20 μl of dissecting medium to a center of a siliconized flat-bottom glass scintillation vial (17 × 52 mm), and the length of the tubules was measured through a drawing tube attached to the microscope as described above. After 5 min of preincubation at 37° C, 20 μl of modified Hanks' solution containing appropriate text hormone(s) or vehicle was added to the incubation medium, and the incubation was continued for an additional 2 min at 37° C. The incubation was terminated by the addition of 50 μl 10% trichloroacetic acid (TCA), and the mixture was vortexed, extracted three times with 0.8 ml of ether saturated with water. The residual aqueous phase was dried at 40 to 45° C, and the vials were kept at −20° C until the assay.

Immediately prior to the assay, 100 μl of 50 mM sodium acetate buffer, (pH, 6.2) was added to each vial, mixed, and the extracts were assayed for cAMP after acetylation by RIA. Recovery of ^{3}H-cAMP added together with TCA was 93 to 102%. Linear relations were observed between the tubular length and cAMP formed by the intact tubules as depicted in Fig. 1. When the extracts of tubules incubated with agonists thus containing varying amounts of cAMP were divided and assayed for cAMP with or without additional 100 fmoles of cAMP, the measured values for added cAMP were 101.8 ± 6.4 ($N = 9$). Values of 105.4 ± 3.7 ($N = 5$) were obtained for the same amount (100 fmoles) of cAMP when assayed without tubular extracts. Values of cAMP obtained in TCA extracts purified further for cAMP by column chromatography followed by the zinc sulfate and barium hydroxide precipitation [10]

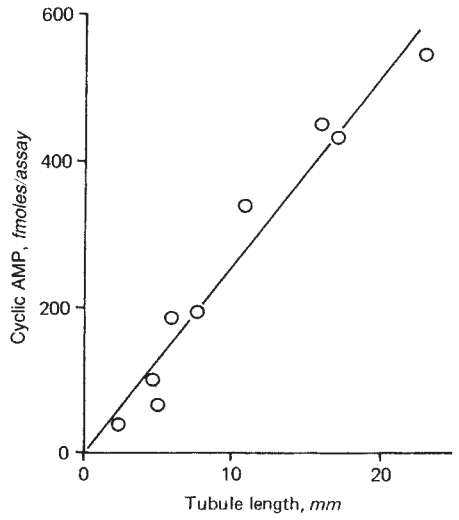

Fig. 1. *Relation between cAMP formed and the tubule length per incubation.* In this experiment, intact proximal convoluted tubules with varying length were incubated for 2 min with 2 U/ml PTH. TCA extracts were dried, resuspended in 125 μl of sodium acetate buffer, and 50 μl aliquots were assayed for cAMP. $Y = 25.7x + 1.5$, $r = 0.975$, $P < 0.001$. See text for details.

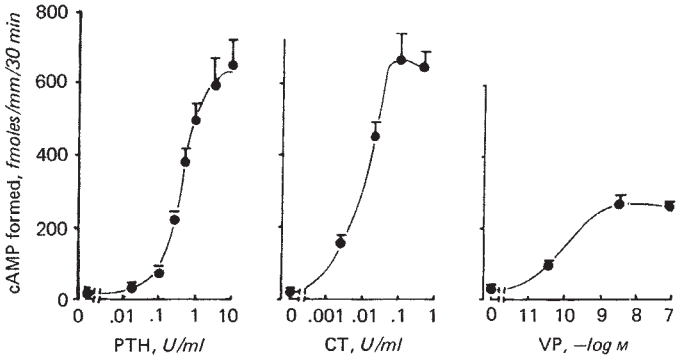

Fig. 2. *Dose-dependent activation by PTH, CT, and VP of adenylate cyclase of rat CTAL.* Each point represents the mean (± SEM) of 4 to 8 incubations.

Table 1. Effects of parathyroid extract (PTE), calcitonin (CT), and vasopressin (VP) on activity of medullary thick ascending limbs of Henle[a]

	cAMP formed, *fmoles/mm/30 min*
Control	47 ± 2 (6)
PTE, 0.5 U/ml	33 ± 2 (5)
CT, 0.1 U/ml	220 ± 19 (4)
VP, 5×10^{-10} M	336 ± 16 (5)

[a] Values are the means ± SEM with the number of incubations in parentheses.

agreed within less than 10% with those without purification procedures.

In separate experiments, the time-course changes in cAMP contents in tubule cells and in the incubation medium were measured. After 5 min of preincubation at 37° C, the test hormone was added as described above. At 2 and 10 min (12 min for PTE experiments) after the hormone addition, 200 μl of ice-cold isotonic saline containing 1.2 mM MIX was added to the incubation vial, which was then centrifuged for 2 min at ×1,000*g* at 4° C. An aliquot (100 μl) of the upper portion of the medium was carefully transferred to another vial, and to both vials, one containing tubules and the incubation medium and another with the medium only, 20 μl of 50% TCA was added. After extracting TCA with ether saturated with water as described above, we dried the extract, resuspended it in 100 μl of 50 mM sodium acetate buffer (pH, 6.2) and assayed it for cAMP by RIA. Intracellular cAMP contents were calculated as the difference between cAMP values in the tubules plus medium and those in the medium. The addition of isotonic saline solution containing MIX was without significant effect on the RIA. Results are expressed as femtomoles of cAMP per millimeter of tubule length.

Results were expressed as the means ± SEM and were analyzed using Student's *t* test.

Results

Dose-response relationship between PTH, CT, and VP, and adenylate cyclase activity of CTAL. As shown in Fig. 2, PTH, CT, and VP all stimulated adenylate cyclase activity of CTAL in a dose-dependent manner. By contrast, PTE was without effect on the enzyme activity of MTAL, whereas CT and VP stimulated it (Table 1). In general, with maximal doses PTH stimulated adenylate cyclase of CTAL to a greater extent than did CT or VP, and VP-stimulated enzyme activity was smallest in this segment. VP, however, exerted a greater enzyme stimulation than did CT in MTAL.

Effects of maximal doses of hormones on adenylate cyclase activity of CTAL. Effects of maximal doses of PTH, CT, and VP in various combinations on adenylate cyclase activity in CTAL are summarized in Table 2. Considerable variations in the magnitude of enzyme stimulation among experiments are observed as already reported [1–6]. With both PTH and CT together, the increments in the enzyme activity were not greater than those with PTH or CT alone. The addition of VP with PTH or CT resulted in enzyme activities not different from those seen with PTH or CT alone. A much smaller magnitude of enzyme activation by VP than that by PTH or CT may have masked the presence of possible additive effects of VP with PTH or CT, however. The addition of three hormones together resulted in the enzyme activity again not different from that seen by PTH, which gave the greatest enzyme stimulation.

Time-course changes in cyclic AMP in CTAL and in the incubation medium. As shown in Fig. 3, tubular cAMP levels rose rapidly in response to hormone addition, and at 2 min only a minor fraction of cAMP was in the incubation medium. At 10 min (or at 12 min for PTE) after the addition of test hormones, cAMP in the incubation medium rose considerably so that total cAMP (tubules plus incubation medium) at 10 min became greater than that at 2 min. Intracellular cAMP levels at 10 min were not different from those obtained at 2 min, however.

Dose-response relation between PTH, CT, VP, and cell cyclic AMP levels of CTAL. The dose-response relationships between PTH, CT, VP, and cell cAMP of intact CTAL are shown in Fig. 4. Increments in cell cAMP depicted in this Fig. 4 are mostly those occurring intracellularly because values represent total cAMP measured at 2 min after hormone addition.

Table 2. Effects of maximal doses of parathyroid hormone (PTH), calcitonin (CT), and vasopressin (VP) on adenylate cyclase activity of rat cortical thick ascending limb of Henle[a]

Additions	Adenylate cyclase activity, *fmoles cAMP formed per mm/30 min*							
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8
None (control)[b]	34.1 ± 4.7 (21)							
PTH, 10 U/ml	834 ± 137 (5)[c]	551 ± 97 (4)[c]	451 ± 34 (8)	—	—	—	817 ± 95 (9)	460 ± 38 (7)
CT, 0.1 U/ml	442 ± 41 (5)	332 ± 127 (5)	—	851 ± 105 (5)	434 ± 65 (5)	723 ± 75 (5)	577 ± 75 (8)	336 ± 52 (5)
VP, 10^{-8} M	201 ± 26 (5)	152 ± 32 (5)	202 ± 48 (6)	357 ± 68 (5)	160 ± 16 (5)	118 ± 17 (5)	—	—
PTH + CT	1052 ± 165 (5)	503 ± 127 (4)	—	—	—	—	754 ± 60 (10)	401 ± 34 (6)
PTH + VP	839 ± 64 (6)	605 ± 162 (5)	502 ± 38 (8)	—	—	—	—	—
CT + VP	652 ± 86 (5)	400 ± 124 (5)	—	824 ± 162 (5)	692 ± 104 (5)	689 ± 84 (5)	—	—
PTH + CT + VP	918 ± 51 (5)	747 ± 155 (6)	—	—	—	—	—	—

[a] Values are the mean ± SEM with the number of incubations in parentheses.
[b] Control values were comparable in these and other experiments and were not measured in every experiment listed here. Thus, all values were combined.
[c] PTH = 5 U/ml.

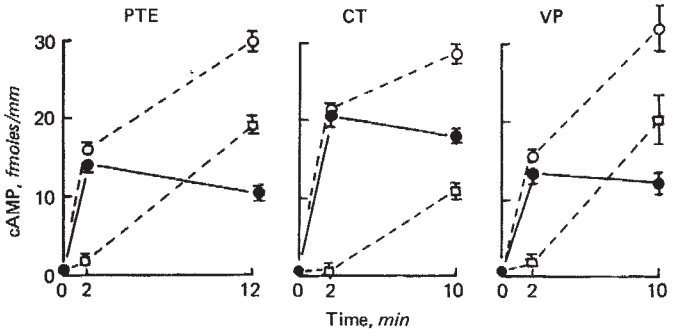

Fig. 3 *Distribution of cAMP in the tubules and incubation medium.* Intracellular cAMP levels (●---●) were calculated from those in the incubation medium plus tubules (○---○) and those in the incubation medium only (□---□). Cyclic AMP levels at 0 time were very low, and only total cAMP could be measured (0.6 ± 0.3, $N = 15$). Concentrations of hormones used were 0.5 U/ml, 0.1 U/ml, and 10^{-8} M for PTE, CT, and VP, respectively. Each point represents the mean (± SEM) of quintuplicate incubations. See text for details.

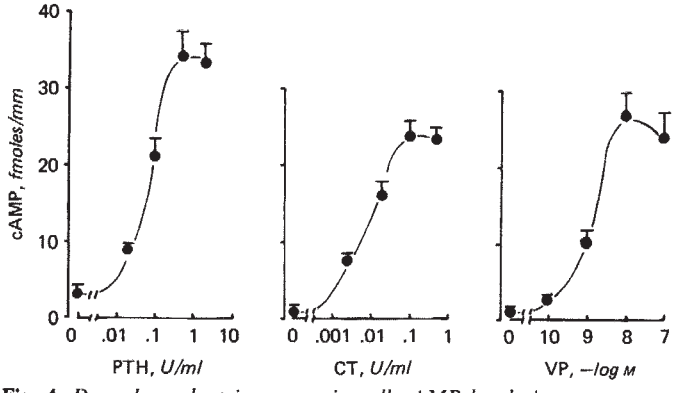

Fig. 4. *Dose-dependent increases in cell cAMP levels in response to PTH, CT, and VP in intact CTAL.* After 5 min of preincubation, the intact CTAL were incubated for 2 min with varying concentrations of test hormones. Each point represents the mean (± SEM) of 4 to 8 incubations.

The doses of hormones that increase cell cAMP to a half maximal and a maximal level were similar to those that activate adenylate cyclase in this nephron segment for CT and VP. But, doses of PTH causing half-maximal stimulation were consistently lower for cell cAMP than they were for adenylate cyclase activity. This difference is apparent in data shown in Figures 2 and 4.

Effect of graded concentrations of 1-methyl-3-isobutylxanthine on cell cyclic AMP levels. Although PTH and CT elicited greater adenylate cyclase activation than VP did, differences in the increments in cell cAMP by these hormones were much smaller compared with the differences observed in adenylate cyclase activation. Indeed, in most experiments, these three peptide hormones increased cell cAMP to comparable levels. Such apparently discrepant effects of agonists on adenylate cyclase and on cell cAMP levels may be due to a possible presence of some metabolic factor in the intact cell preparation, such as ATP availability for adenylate cyclase, which may become limiting in cell cAMP accumulation. To evaluate such a possibility, we examined increments in cell cAMP in the absence and in the presence of graded inhibition of phosphodiesterase activity. As shown in Table 3, increments in cell cAMP in response to hormone addition were minimal in the absence of a phosphodiesterase inhibitor, MIX. In the presence of either 0.12 or 1.2 mM MIX, cell cAMP rose to comparable levels in response to maximal doses of PTH, CT, or VP. But, cAMP levels rose to higher levels with 1.2 mM MIX than they did with 0.12 mM MIX.

Effects of various combinations of maximal doses of hormones on cell cyclic AMP. As shown in Tables 3 and 4, levels of cell cAMP in response to these hormones added in various combinations were not greater than those to each hormone added separately. A recent study by Bailly et al [8] showed the presence of glucagon (GL) -sensitive adenylate cyclase in TAL of rat kidney, and therefore, the effects of GL on cell cAMP were also examined. As shown in Table 5, no additive effects were observed in increments in cell cAMP in response to PTH, VP, and GL in CTAL; effects of VP and GL on cell cAMP in MTAL were also not additive (Table 5).

Discussion

The present study confirmed and extended previous observations by Morel, Chabardes, and Imbert [7]. Maximal enzyme activation of CTAL was obtained at hormone concentrations of 5 U/ml, 0.1 U/ml, and 10^{-8} M for PTH, CT, and VP, respectively. The magnitude of enzyme activation of CTAL with maximal hormone concentrations was greatest with PTH followed by CT, then VP. There is a clear distinction in hormone responsiveness of adenylate cyclase between cortical and medullary

Table 3. Effects of MIX on cell cyclic AMP levels in response to parathyroid hormone (PTH), calcitonin (CT), and vasopressin (VP)[a]

Additions	Cell cyclic AMP, *fmoles/mm*		
	0 mM MIX	0.12 mM MIX	1.2 mM MIX
None (control)	0.6 ± 0.2 (10)	NT[b]	NT[b]
PTH, 10 U/ml	3.5 ± 1.0 (5)	23.5 ± 3.7 (5)	51.1 ± 1.7 (3)
CT, 0.1 U/ml	1.3 ± 0.7 (5)	27.2 ± 3.2 (5)	45.4 ± 10.4 (3)
VP, 10^{-8} M	3.2 ± 0.5 (5)	21.2 ± 1.6 (5)	45.6 ± 11.2 (3)

[a] Values are the mean ± SEM with the number of incubations in parentheses. Isolated cortical thick ascending limbs of Henle were incubated in the presence or absence of MIX for 5 min at 37° C. Then hormones were added at final concentrations indicated, and the incubation was carried out for an additional 2 min. The results represent data obtained in the CTAL dissected from the same rat. See text for details.

[b] Not tested in these experiments. In other experiments, however, values with 1.2 mM MIX were always less than 10 fmoles/mm, and with 0.12 mM MIX less than 4 fmoles/mm.

Table 4. Effects of maximal doses of parathyroid hormone (PTH), calcitonin (CT), and vasopressin (VP) on cyclic AMP levels in intact cortical thick ascending limbs of Henle of rat kidney[a]

Additions	Cell cyclic AMP, *fmoles/mm*			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
None (control)[b]	0.6 ± 0.3 (15)			
PTH, 10 U/ml	15.3 ± 1.5 (4)	36.7 ± 3.8 (4)	—	—
CT, 0.1 U/ml	13.0 ± 1.7 (4)	29.4 ± 1.2 (5)	15.8 ± 1.2 (5)	15.8 ± 1.6 (5)
VP, 10^{-8} M	11.5 ± 0.9 (4)	30.3 ± 2.6 (5)	12.2 ± 0.9 (5)	26.2 ± 2.5 (5)
PTH + CT	12.2 ± 0.5 (4)	28.6 ± 2.9 (5)	—	—
PTH + VP	14.4 ± 1.2 (4)	27.5 ± 1.5 (5)	—	—
CT + VP	—	—	13.6 ± 0.8 (5)	26.7 ± 2.4 (8)
PTH + CT + VP	13.5 ± 1.5 (4)	38.1 ± 2.5 (5)	—	—

[a] Values are the mean ± SEM with the number of incubations in parentheses.

[b] Control values were comparable in these and other experiments and were not measured in every experiment listed here. Thus, all values were combined.

portions of TAL; in MTAL, the magnitude of enzyme activation was greater with VP than with CT, and PTH was without effect.

Data shown in Table 2 suggest that PTH and CT may stimulate the same adenylate cyclase moieties because the addition of maximal doses of both hormones together demonstrate a similar stimulation of enzyme activity that obtained in each hormone added separately. The data are in accord with those reported by Morel et al [11]. A much smaller magnitude of enzyme activation by maximal doses of VP makes the presence of additivity with VP together with PTH and/or CT inconclusive. Thus, whether VP also stimulates the same adenylate cyclase moieties responsive to PTH and CT is not clear. The presence of rather large intra- and inter-experiment variation coefficients in the present study is similar to that observed by Morel et al [1–6], but explanations for such variations are not immediately clear. It is possible that some conditions in individual animals or in experimental procedures may affect hormone responsiveness. But, in the present study we did not attempt to subject rats to specific pretreatments such as thyroparathyroidectomy, dehydration, and so on, prior to the experiments, procedures that might affect hormone-sensitive adenylate cyclase activities.

In the present study, we were able to detect changes in cell cAMP in intact single nephron segments dissected from rat kidney. Increments in cell cAMP at 2 min after the hormone addition were limited almost exclusively to the intracellular spaces. At 10 min after the hormone addition, however, up to 64% of total cAMP measured in the tubules and incubation medium represents extracellular cAMP (Fig. 3).

Table 5. Effects of glucagon on cell cyclic AMP levels in intact cortical and medullary thick ascending limbs of Henle of rat kidney[a]

Additions	Cell cyclic AMP, *fmoles/mm*		
	Exp. 1	Exp. 2	Exp. 3
Cortical TAL			
None (control)	4.4 ± 0.8 (4)	7.7 ± 0.2 (4)	2.8 ± 4 (3)
Glucagon, 10^{-6} M	53.3 ± 6.9 (4)	48.3 ± 4.8 (4)	38.0 ± 2.9 (4)
VP, 10^{-8} M	39.1 ± 3.8 (4)	—	38.9 ± 2.3 (4)
PTH, 10 U/ml	34.4 ± 1.5 (4)	36.2 ± 3.0 (4)	—
GL + VP	56.2 ± 6.3 (4)	—	47.5 ± 2.2 (4)
GL + PTH	38.5 ± 6.3 (4)	46.4 ± 4.2 (4)	—
Medullary TAL			
None (control)	2.0 ± 0.5 (3)	2.2 ± 1.1 (3)	
Glucagon, 10^{-6} M	14.7 ± 2.3 (4)	16.0 ± 2.0 (4)	
VP, 10^{-8} M	15.2 ± 1.3 (4)	21.2 ± 1.1 (4)	
GL + VP	15.3 ± 1.4 (4)	23.2 ± 3.1 (4)	

[a] Values are the means ± SEM, with the number of incubations in parentheses.

In contrast to greater stimulation of adenylate cyclase by maximal doses of PTH and CT than that of VP, the magnitude of increments in cell cAMP by maximal doses of PTH, CT, and VP was similar. The reason for this apparent discrepant effect of these hormones on adenylate cyclase activation and on rises in cell cAMP is not clear. In contrast to the assay of adenylate cyclase activity where the substrate, ATP, is provided in far excess of the reaction product, cAMP, some metabolic factors may limit the production of cAMP in intact cells despite maximal stimulation of adenylate cyclase. Stated in another

way, maximal increments in cell cAMP in the presence of maximal hormone concentrations may be limited by some factors other than adenylate cyclase activity. Such a factor may include availability of ATP for adenylate cyclase. Although levels of cell ATP are far in excess of cAMP when measured in the whole kidney (approximately 1000 to 1 [12–14]), it is possible that ATP available for adenylate cyclase in CTAL may easily become limited under hormonal stimulation. But, as shown in Table 3, increments in cell cAMP in response to maximal concentrations of PTH, CT, and VP were similar in both the presence and the absence of phosphodiesterase inhibitor. Moreover, in the presence of 1.2 mM MIX, these cells can produce as much as 40 to 50 fmoles/mm cAMP in 2 min; that is, this much ATP must be available for cAMP production. Nonetheless, all three hormones could increase cell cAMP up to 30 fmoles/mm in 2 min in the presence of a lower concentration of the inhibitor. These data suggest that even under the condition where ATP is unlikely to be a limiting factor in cell cAMP production, maximal doses of PTH, CT, and VP increased cell cAMP to comparable levels. We do not know at present the reason for the apparent discrepant effects of maximal doses of PTH, CT, and VP on adenylate cyclase activity and on cell cAMP contents.

The addition of maximal doses of PTH, CT, VP, and GL in various combinations clearly failed to increase cell cAMP beyond those observed with each hormone added separately (Tables 3, 4, and 5). Also, the effects of VP and GL on cell cAMP of intact MTAL were not additive (Table 5). If these hormones are acting on separate cell groups in TAL, one would expect additive effects of maximal doses of these hormones on cell cAMP contents. Thus, the lack of additive effects on cell cAMP strongly suggests that these hormones act on the same cell group in this nephron segment. Available morphologic evidence suggests that the cell population in CTAL primarily consists of one major cell type characterized by numerous and prominent microvilli on the luminal surface [15].

If these four different peptide hormones are to act on the same cells in CTAL, one might expect that they may have qualitatively similar effects on the CTAL functions, assuming such tubular functional responses are mediated by cAMP. Similar conclusions could also be drawn for the actions of VP and GL on MTAL, as both hormones seem to act on the same cells in MTAL. At present, the physiologic significance of adenylate cyclase in CTAL sensitive to these hormones is not known. Recent studies using the isolated tubule perfusion technique demonstrated stimulation of calcium reabsorption in CTAL by PTH and dibutyryl cAMP [16–18]. If such effects of PTH on calcium transport are mediated by cAMP, one might expect that CT, VP, and GL may also show qualitatively similar effects on calcium transport in this nephron segment. It is of note that these perfusion studies with isolated CTAL are performed in the rabbit nephron and that the present results were obtained in the rat nephron. Nonetheless, in the CTAL of rat and rabbit kidneys, PTH, CT, and VP all can stimulate adenylate cyclase. Thus, it is of interest to examine whether these peptide hormones have similar effects on tubular function in CTAL of rabbit and rat kidneys. If these hormones demonstrate distinctly different or even opposite effects on CTAL functions, then it follows that such tubular responses are not mediated solely by cAMP and that some other cellular events must be operative as a mechanism of hormone action.

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